ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Prevalence of Multidrug Resistant Enterobacteriaceae and Extended Spectrum β Lactamase Producing *Escherichia Coli* in Urinary Tract Infection

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ABSTRACT

Urinary tract infection is major health problem in Nepal. Considering, the majority of infection cases caused by Enterobacteriaceae with *Escherichia coli* being a major pathogen, the present study was carried out to investigate and identify the Multidrug Resistant pattern of Enterobacteriaceae and Extended Spectrum β-lactamase producing *Escherichia coli* so that effective strategy for the urinary tract infection treatment can be achieved. A total of 650 specimens were processed at National Public health laboratory (NPHL), Kathmandu Nepal between November 2010 to April 2011. Extended Spectrum β- lactamase screening among multidrug resistant isolates was done using Ceftriaxone, Aztreonam, Cefotaxime, Ceftazidime and Cefpodoxime followed by confirmation using MASTDISCTMID D6. Data analysis was done by SPSS 16 software. Enterobacteriaceae remain predominant of the total isolates (78.7%) of which, *Escherichia coli* was the most common organism with (64.0%) followed by *Klebsiella* species (17.9%).Among the total 42 multidrug resistant *Escherichia coli* subjected for Extended Spectrum β-lactamase screening test 18(31.57%) were confirmed as Extended Spectrum β- lactamase producer by at least one combined disk assay. All the isolates were sensitive to Imipenem (100%) followed by Meropenem (94.44%).

Keywords: *Escherichia coli,* Enterobacteriaceae, Extended Spectrum β- lactamase, Nepal

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ISSN: 0975-8585

INRODUCTION

Urinary tract infection represents one of the commonest bacterial infections. The Enterobacteriaceae are the most frequent pathogen detected, causing 84.3% urinary tract infection[1].The pathogen causing UTI are almost always predictable, with *E.coli* as the primary etiological agent among both inpatients and outpatients. Other common gram negative organism causing UTI are *Klebsiella* spp, *Enterobacter*, *Proteus* and *Citrobacter* spp. Among gram positive, *Streptococci* and *Staphylococcus saprophyticus* are significantly associated with the disease [2].

Infections by Enterobacterial isolates resistant to extended spectrum cephalosporin have become a serious problem worldwide [3]. MDR Enterobacteriaceae has been frequently reported from different parts of the world as an emergence of treatment problem. Antibiotics given empirically without proper antibiotic susceptibility testing are one of the major causes for the development of MDR. So, to ensure appropriate therapy, current knowledge of the organism that causes UTI and their antibiotic susceptibility is mandatory [4].

In human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics. The volume of antibiotics prescribed is also a major factor in increasing rates of bacterial resistance rather than compliance with antibiotics. Besides this causes, poor hand hygiene by hospital staff has been associated with the spread of resistant organism. In some countries, antibiotics are sold over counter without a prescription which compounds the problem [5]. The ESBL problem exists in our country so this research has been performed with a view to provide antibiotic guidelines which will aid in minimizing the problem along with stringent infection control practices.

METHODS

Study Population and Sample Collection

The present study was conducted at National Public Health Laboratory, Teku. The study was carried out from November 2010 to April 2011. During this period, a total of 650 urine samples from patients suspected of UTI were collected and processed according to the standard laboratory methods. A self-structured questionnaire form was filled to achieve information on socioeconomic status and hygienic behavior interviewing each participant.

Processing of the Samples

Semi-quantitative method was used for the culture of urine specimen. In this technique, the standard loop having loop diameter 4mm was used. By using this loop, the approximate number of bacteria per ml of urine can be estimated which contains approximately 0.001 ml urine. The urine was mixed well by inverting the container several times. Then by using a sterile calibrated wire loop, a loopful of urine was inoculated on blood agar and MacConkey agar plate and the plates were incubated aerobically at 37°C for 24 to 48 hours.

Identification of Isolates

Bacterial isolates were identified by standard microbiological techniques as described in the Bergey's manual of systemic bacteriology which involves colony characterization, cell morphology and biochemical tests.

Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing of the isolates against antimicrobial disks was done by modified Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute [6].

Screening and Confirmation for ESBL producing Isolates

The screening agents, viz. Aztreonam (30µg), Ceftriaxone (30µg), Cefpodoxime (10µg), Ceftazidime (30µg) and Cefotaxime (30µg) (Mast Diagnostics, UK) were placed onto the inoculated media and incubated at 37C for 18-24 hours.Isolates showing Aztreonam <27 mm, Cefotaxime <27 mm, and Ceftriaxone <25 mm were suspected as possible ESBL producers. All the processed bacterial isolates were then subjected to phenotypic confirmatory test using Combined Disks (CD) Assay; an increase in zone diameter of >=5mm in the presence of Clavulanic acid from any or all of the combination discs confirmed the isolates as ESBL producers.

Statistical Analysis

The chi-square was used as per need to determine significant association between different attributes for the causation of UTI.

RESULTS

Among the 113 (17.38%) isolates, 102(90.26%) were Gram negative bacteria. Enterobacteriaceae were the major one with *E. coli* the most frequently isolated species. Among the 11 Gram positive isolates, *Staphylococcus aureus* was the most predominant with 5 (45.45%) isolates (Table 1).

The age group of 21-30years had the maximum growth and the least growth was from the patients of age group above 80 years. Among the isolates, higher number of pathogens were isolated from sample of female patients (56.64%) compared to male (43.36%).

Amikacin and Nitrofurantoin were found to be the most effective drug for Enterobacteriaceae family and Amoxycillin was found to be the least effective drug .The antibiotic susceptibility pattern of *E. coli* showed that Amikacin was the most effective drug which was followed by Nitrofurantoin. Amoxicillin was found to be the least effective drug (Table 2).

E. coli showed the predominant number of MDR isolates (73.68%) and 43.75% of *Klebsiella* spp were found to be MDR strains (Table 3). Higher rate of MDR was found in female patients compared to male patients. The association of MDR and non-MDR strains in males and females was found to be statistically significant (P=0.017). The results are shown in table 4.

Of the total 89 isolates of Enterobacteriaceae, 57 were *E. coli* and out of them, 42 were MDR. Among them, 26 were found to be screen positive for ESBL and among the screen positive 18 were confirmed as ESBL producers (Table 5). ESBL *E.coli* was most commonly isolated in the older age i.e. above 60 years in both genders and was not isolated in the age group of less than 21 years and greater than 81 years. The age and sex distribution of the ESBL is shown in the tables 6. Antibiogram of ESBL producing *E. coli* towards Carbapenems showed that imipenem was found to be more effective than Meropenem towards ESBL *E. coli* isolates (Table 7).

DISCUSSION

Contrary to the earlier studies, this study showed relatively higher number of male patients requesting urine culture. However, higher percentage of significant growth was found in female patients and this finding is stastically significant (P=0.040) . Same was true in the case of MDR strains. Uethral opening in females, short urethra, complicated physiology especially during pregnancy can be the considered as reason. Similar results were seen in the earlier studies [8-10].

The age group of 21-30 was found predominant (28.46%) for requesting urine culture. The significant growth from urine sample was predominant (25.66%) in age group 21-30 which is followed by the age group 31-40. Age group 21-30 is sexually active that may justify this finding. High prevalence of UTI (24.48%) in old age male subjects of age group (61-70 years) may be due to different conditions like prostatitis, diabetes and weak immune status.

Gram negative isolates (90.26%) were the predominant pathogens of the urinary tract infections with Enterobacteriaceae (78.76%) as the major one. Of the 57 *E. coli* isolates, 42 (73.68%) were multidrug resistant, *E. coli* followed by *Klebsiella* spp and Enterobacteriaceae other than *E.coli* and *Klebsiella* spp accounted for (50.00%) resistance. These results resembled the outcomes of previous studies [9, 10].

The high level of drug resistance seen among *E. coli* is mediated by β-lactamases, which hydrolyze the β-lactam ring inactivating the antibiotic, the classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of Gam-negative rods[11]. Mutations at the target site i.e. *gyrA*, which is a gyrase subunit gene, and *parC*, which encodes a topoisomerase subunit, confer resistance to fluoroquinolones[12]. *E. coli* have special virulent properties contributing to their being a major uropathogen throughout the world. *E. coli* can bind to the glycoconjugate receptor (Gal alpha1-4 Gal) of the uroepithelial cells of human urinary tract such that it can initiate infection itself. *E.coli* is isolated in 90% of Urinary tract

infections and strains are characterized by unique virulence determinant, the p pilus (Gal-Gal) [13].

Majority (64.04%) of member of Enterobacteriaceae were found to be MDR. The association of MDR and non-MDR strains in males and females was found to be statistically significant ($P<0.05$). The high degree of resistance could be explained by the fact that drugs are easily available without doctor's prescription from pharmacy and in developing countries like Nepal self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates. Since patient only think of going to the hospitals when they are unable to treat themselves. Expired antibiotics, self-medication counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [21].

In this study, 42 multi-drug resistance E.coli were screened for ESBL production using the CLSI recommended screening agents viz. Ceftazidime, Cefotaxime, and Ceftriaxone, of which 23(54.76)% were classified as possible ESBL producers. Among these cephalosporins, resistance was very high to ceftriaxone,cefotaxime and ceftazidime this finding is in line with other studies[14].

In this study, 18 (31.57%) *E.coli* was confirmed as ESBL producer. In a similar study, Buda (2010) and Bomjan (2005) reported 25.32% and 27.03% ESBL producing *E. coli* respectively. In contrast to these studies, high prevalence (>50%) of ESBL producing *E.coli* has been reported in other studies [15, 16]. In another study, 7.2% 2.5% and 18.5% ESBL producing *E. coli* respectively [17, 20].Maximum number of ESBLs (around 50%) was isolated in the older age group of ≥61 years in both male and female patients this may be due to concomitant disease process and recent prior antibiotic treatments [19]. The reason for high prevalence of ESBL *E.coli* in older age female may be the lack of estrogen, which is essential to maintain the normal acidity of vaginal fluid [20]

In the present study, all the 18 ESBL producing *E.coli* was sensitive to imipenem. Similar findings have also been reported by other studies. Similarly, 98.3% *E.coli* susceptible to imipenem. In case of Meropenem 94.44% *E.coli* were found susceptible. Similar findings have also been reported by other workers [21].

CONCLUSION

In the present study, the increasing pattern of the drug resistance seen among ESBL producers was All the ESBL producers were resistant to five or more of the most commonly used antibiotics and was comparable to findings of other studies. ESBL-producing strains are creating significant therapeutic problems since these pathogens are resistant to a wide range of ß-lactams, including third generation cephalosporins as well as have potential for plasmid mediated Quinolone and carbapanem resistance is creating significant therapeutic problems. As indicated by the present finding together with previous findings, it appears to be necessary to

ISSN: 0975-8585

include ESBL detection in routine laboratory practice so as to limit the rapid spread of ESBL-producing organisms.

Table 1: Isolates from Urine Sample

Organism	No. of Isolates (%)	% of Total Isolates
Gram Positive Bacteria		
Staphylococcus aureus	5 (45.45)	4.42
Staphylococcus saprophyticus	2 (18.18)	1.77
Enterococcus spp	4 (36.36)	3.54
Sub-total	11 (100)	9.73
Enterobacteriaceae		
Escherichia coli	57(64.04)	50.44
Klebsiella spp	16 (17.97)	14.15
Citrobacter spp	6 (6.74)	5.30
Enterobacter spp	5 (5.61)	4.42
Proteus spp	3 (3.37)	2.65
Serratia spp	1 (1.12)	0.88
Salmonella paratyphi A	1 (1.12)	0.88
Sub-total	89 (100)	78.76
Gram Negative		
Non-Enterobacteriaceae		
Acinetobacter spp	6 (46.15)	5.31
Pseudomonas aeruginosa	3 (23.07)	2.65
Alcaligeness spp	4 (30.76)	3.54
Sub-total	13 (100)	11.50
Total Isolates	**113 (100)**	

ISSN: 0975-8585

Table 2: Antibiotic susceptibility profile of *E.coli* (n=57)

S.N.	Antibiotics	Susceptibility Pattern					
		Susceptible		Intermediate		Resistant	
		No.	%	No.	%	No.	%
1.	Amoxycillin	7	12.2	0	0.0	50	87.7
2.	Nitrofurantoin	53	92.9	0	0.0	4	7.0
3.	Cotrimoxazole	29	50.8	0	0.0	28	49.1
4.	Norfloxacin	34	59.6	0	0.0	23	40.3
5.	Ciprofloxacin	20	35.0	3	5.2	34	59.6
6.	Ofloxacin	29	50.8	3	5.2	25	43.8
7.	Cefotaxime	31	54.3	0	0.0	26	45.6
8.	Ceftriaxone	35	61.4	0	0.0	22	38.5
9.	Ceftazidime	36	63.1	0	0.0	21	36.8
10.	Amikacin	54	94.7	0	0.0	3	5.2
11.	Gentamicin	37	64.9	0	0.0	20	35.0
12.	Chloramphenicol	41	71.9	7	12.2	9	15.7

Table 3: Distribution of MDR pathogens of Enterobacteriaceae family

Organisms	Total number	MDR Strains	MDR %
E. coli	57	42	73.68
Klebsiella spp	16	7	43.75
Enterobacteriaceae other than *E.coli* and *Klebsiella spp*	16	8	50.00
Total	**89**	**57**	**64.04**

ISSN: 0975-8585

Table 4: Gender wise distribution of MDR Enterobacteriaceae

Gender	Total isolates	MDR Strains	MDR %	P-value
Male	38	19	50.00	P=0.017
Female	51	38	74.50	
Total	**89**	**57**	**64.04**	

Note: P value calculated using Chi-square test at 5% level of significance and d.f.=1

Table 5: Profile of Urine samples and Status of MDR Enterobacteriaceae and ESBL *E.coli* Isolates

Specimen	Total Enterobacteriaceae		*E.coli* isolates		MDR *E.coli*		Suspected ESBL	ESBL producers
			No.	%	No.	%		
Urine (650)	89	78.76%	57	64.04%	42	73.6 %	23 (40.35)%	18 (31.57%)

Table 6. Age and Gender wise Distribution of *E. coli* (ESBL producing)

Age group	Male		Female		Total ESBL	Total ESBL (%)
	Total	ESBL	Total	ESBL		
≤10	1	0	1	0	0	0.00
11-20	1	0	2	0	0	0.00
21-30	2	0	8	2	2	11.11
31-40	1	0	10	2	2	11.11
41-50	3	1	4	1	2	11.11
51-60	5	1	1	0	1	5.55
61-70	9	5	1	1	6	33.33
71-80	1	0	6	5	5	27.77
≥81	0	0	1	0	0	0.00
Total	**23**	**7**	**34**	**11**	**18**	

ISSN: 0975-8585

Table 7: Antibiogram of ESBL producing *E. coli* towards Carbapenems

S.N.	Antibiotics	Total	Susceptibility Pattern					
			Susceptible		Intermediate		Resistant	
			No.	%	No.	%	No.	%
1.	Imipenem	18	18	100	0	0.00	0	0.00
2.	Meropenem	18	17	94.44	1	5.55	0	0.00

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